Journal of Medical Virology 48:151–156 (1996)

Molecular Epidemiology of Adenovirus Acute Lower Respiratory Infections of Children in the South Cone of South America (1991–1994)

Adriana E. Kajon, Alicia S. Mistchenko, Cristina Videla, Maria Hortal, Göran Wadell, and Luis F. Avendaño

Departamento de Microbiologia, Facultad de Medicina, Universidad de Buenos Aires (A.E.K.), Laboratorio de Virologia, Hospital de Niños "Ricardo Gutierrez" (A.S.M.), and CEMIC (C.V.), Buenos Aires, Argentina, Department of Virology, University of Umeå, Umeå, Sweden (A.E.K., G.W.), Departamento de Laboratorios de Salud Pública, Montevideo, Uruguay (M.H.), Facultad de Medicina, Universidad de Chile, Santiago, Chile (L.F.A.)

A collection of 165 adenovirus strains isolated from nasopharyngeal aspirates of children hospitalized for acute lower respiratory infection in Argentina, Chile, and Uruguay between 1991 and 1994 was studied by restriction enzyme analysis (work performed in the Department of Virology, University of Umeå). Of the isolates, 71% (n = 117) were identified as members of subgenus B. Of these, 101 (61.2%) corresponded to genome type 7h, four (2.4%) to genome type 3p2, four (2.4%) to genome type 11a, one (0.6%) to genome type 7b, and one (0.6%) to genome type 7c. Two isolates that were neutralized as serotype 3 and four isolates that were neutralized as serotype 7 exhibited novel BamHI cleavage profiles corresponding to three new genome types denominated 3x, 7i, and 7j.

Subgenus C members represented 28.5% of all typed isolates. Five different genome types of Ad1, seven genome types of Ad2, and three genome types of Ad5 were identified of, which two, two, and one, respectively, were found to correspond to new DNA variants. Only one isolate (0.6%) corresponded to Ad4 of subgenus E. Ad7h was isolated from 17 of the 18 fatal cases recorded among the patients included in the study.

KEY WORDS: restriction enzyme analysis, severe pneumonia

INTRODUCTION

Acute respiratory infections (ARI), predominantly from viruses, are the major cause of morbidity and mortality in infants and young children throughout the world. They have a particular impact on developing countries where 4 million ARI-related deaths are estimated to occur each year among the susceptible population under the age of 5 years [Lewoski, 1986; Campbell, 1992]. In those countries of South America where the viral etiology of acute respiratory infection requiring hospitalization has been investigated, adenovirus was found to be the second major virus to infect the respiratory tract of children after respiratory syncytial virus [Vicente Suarez et al., 1988a,b; Avila et al., 1989, 1990; Nascimento et al., 1991].

Since 1984, we have been studying the molecular epidemiology of adenoviruses associated with severe acute lower respiratory infection requiring hospitalization in Argentina, Chile, and Uruguay [Kajon and Vicente Suarez, 1990; Kajon et al., 1993, 1994; Kajon and Wadell, 1992, 1994]. With the aim of completing a 10-year period of continuous surveillance, we studied a collection of 165 adenovirus strains isolated in the south cone of America between 1991 and 1994.

MATERIALS AND METHODS

Patients and Samples

All adenovirus strains included in this study were isolated from the respiratory secretions of children <5 years of age hospitalized for acute lower respiratory disease in the cities of Buenos Aires, Argentina, Santiago, Chile, and Montevideo, Uruguay, between April 1991 and November 1994. Nasopharyngeal aspirates were obtained on admission or within the first 3 days of hospitalization. Work was performed in the Department of Virology, University of Umeå (Umeå, Sweden).

Viral Isolation

Adenovirus isolates were obtained by inoculation of tubes of HEp-2 or A-549 cells with clinical specimens. Cells were grown in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 5% fetal calf serum (FCS), glutamine, and antibiotics and maintained with DMEM supplemented with 2% FCS. Viral stocks

Accepted for publication August 20, 1995

Address reprint requests to Dr. Adriana E. Kajon, Department of Virology, University of Umeå, S-901 85 Umeå, Sweden.

TABLE I. Characteristics of the Patient Population With Respiratory Adenovirus Infection

Factor	%
Clinical diagnosis	
Pneumonia	51.9
Pneumonia + wheezing bronchitis	11.7
Bronchiolitis	31.2
Bronchitis	3.9
Pertussislike syndrome	1.3
Sex	
Male	67.5
Female	32.5
Age	
0–5 months	48.3
6–11 months	34.2
12–23 months	12.8
24–60 months	4.7
Fatal cases	10.9 (n = 18)

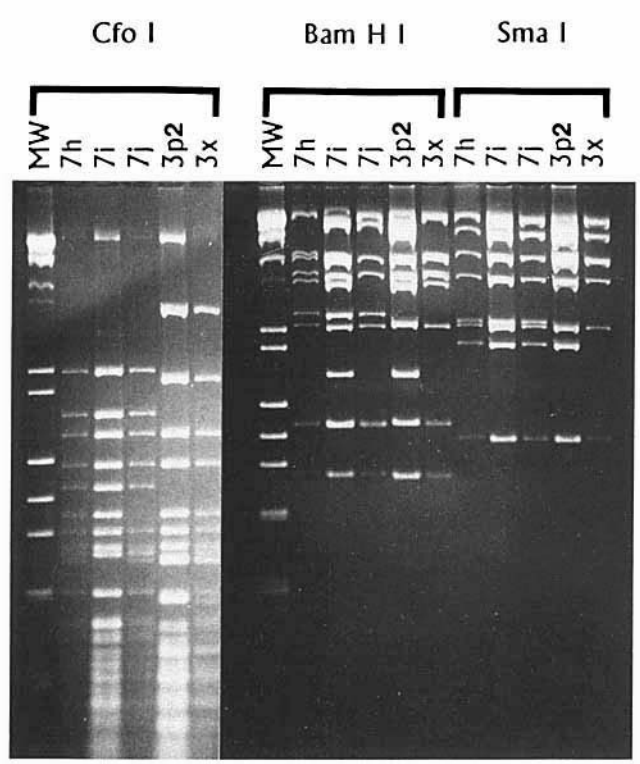

Fig. 1. Cfo I, Bam HI, and Sma I restriction profiles of genome types 7h, 7i, 7j, 3p2; and 3x. **MW:** molecular weight marker (λ DNA digested with Hind III + ΦX DNA digested with Hae III).

of each isolate were grown in A-549 cells and kept frozen at −70C for further analysis.

DNA Extraction

Each isolate was used to inoculate confluent monolayers of A-549 cells in 75 cm^2 plastic flasks. When extensive cytopathic effect was evident (usually 48–72 hr post infection), intracellular viral DNA was extracted by a modification of the method described by Shinagawa et al. [1983].

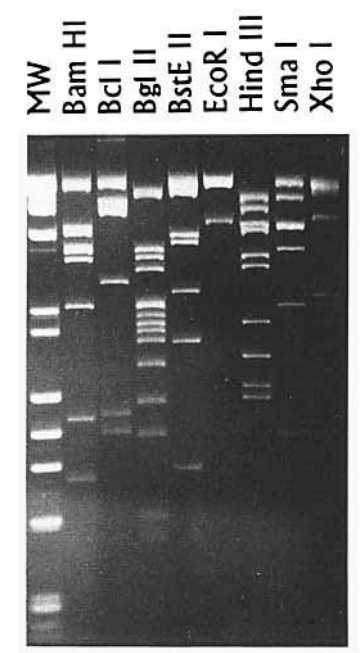

Fig. 2. Restriction profiles of genome type 3x. **MW:** molecular weight marker (λ DNA digested with Hind III + ΦX DNA digested with Hae III).

Restriction Enzyme Analysis

Aliquots containing 1–2 μg of viral DNA were digested with 10–15 units of different endonucleses under conditions specified by the manufacturer (Promega, Madison, WI). Enzyme digests were loaded onto 0.8–1.2% horizontal agarose gels and run at 50 volts for 16 hr in TBE buffer (0.089M Tris-Borate, 0.002 M EDTA, pH 8). Restriction profiles were visualized by staining with 0.25 μg/ml of ethidium bromide and inspection on a U.V. transilluminator. Stained gels were photographed on Polaroid Land film type 55 or 57.

RESULTS

A total of 165 epidemiologically unrelated adenovirus strains isolated from nasopharyngeal secretions of children <5 years of age, hospitalized for acute lower respiratory disease between April 1991 and November 1994 in Argentina (n = 102), Chile (n = 48), and Uruguay (n = 15), were typed and characterized by restriction enzyme analysis. The collection studied comprised 60 isolates obtained in 1991, 42 isolates obtained in 1992, 39 isolates obtained in 1993, and 24 isolates obtained in 1994. The characteristics of the patient population and associated clinical syndromes are shown in Table I. Pneumonia was the most frequent main diagnosis (51.2%), followed by bronchiolitis (31.2%). Eighteen fatalities (10.9%) were recorded among the cases studied.

Typing was carried out initially by digestion of viral DNA with endonucleases Bam HI and Sma I. Additional enzymes were used for a more detailed analysis. Of the isolates, 71% (n = 117) were identified as mem-

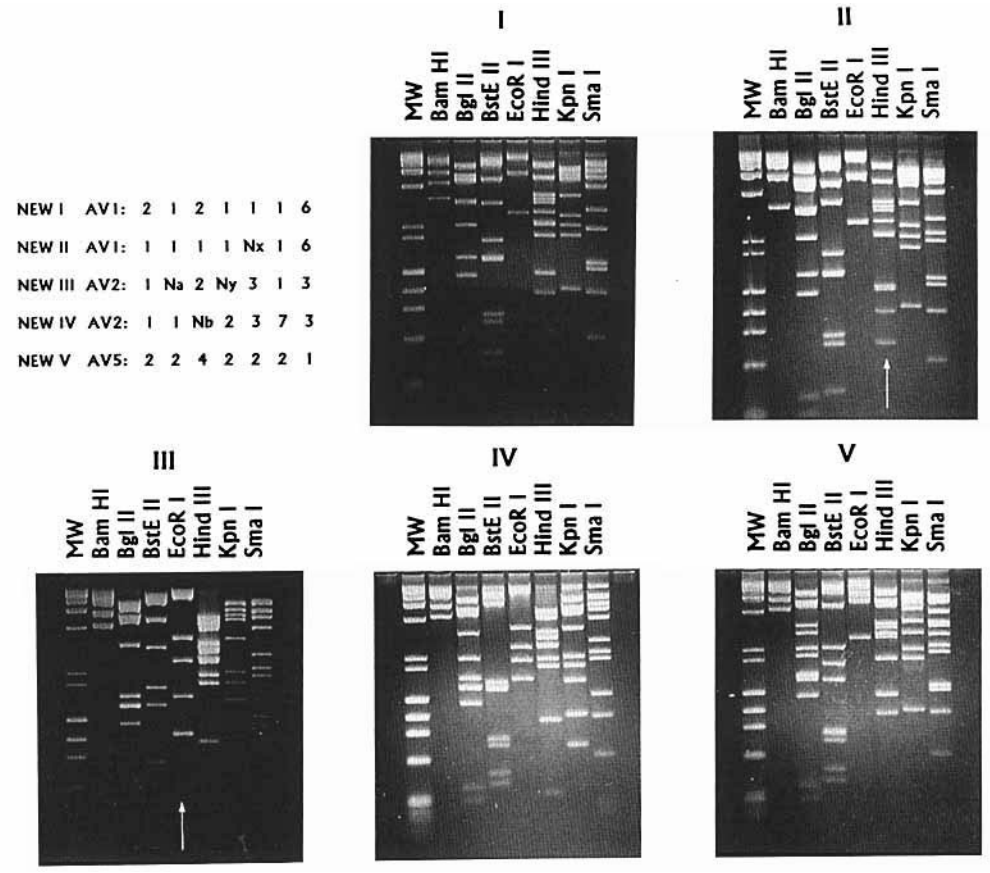

Fig. 3. Restriction profiles of new genome types (I-V) of Ad1, Ad2, and Ad5. **MW:** molecular weight marker (λ DNA digested with Hind III + ΦX DNA digested with Hae III). Arrows indicate new restriction profiles.

TABLE II. Enzyme Codes of Genome Types Belonging to Subgenus C

Type	Genome type	Enzyme code							Number of isolates
		BamHI	BgI II	Bst EII	EcoRI	Hind III	Kpn I	Sma I	
AV1	D1	1	1	1	1	1	1	1	6
	D7	2	1	2	1	1	1	1	4
	D10	2	1	3	1	1	1	1	1
	NEW I	2	1	2	1	1	1	6	1
	NEW II	1	1	1	1	Nx[a]	1	1	1
AV2	D5	1	1	2	2	3	1	3	2
	D7	1	1	2	1	1	1	4	2
	D25	1	1	2	2	3	1	6	2
	D36	1	1	2	2	2	1	6	2
	D63	1	Na[b]	2	2	3	1	3	6
	NEW III	1	Na[b]	2	Ny[a]	3	1	3	5
	NEW IV	1	1	Nb[b]	2	3	7	3	2
AV5	D1	1	1	1	1	1	1	1	1
	D38	2	2	2	2	2	2	1	8
	NEW V	2	2	4	2	2	2	1	4

[a]Restriction profiles shown in Figure 3.
[b]Restriction profiles BgI II Na and Bst EII Nb have been described previously [Kajon et al., 1993].

bers of subgenus B. Of these, 111 were found to be previously characterized genomic variants: 101 (61.2%) corresponded to genome type 7h [Niel et al., 1991], four (2.4%) to genome type 3p2 [Li and Wadell, 1988a], four (2.4%) to genome type 11a [Li et al., 1991], one (0.6%) to genome type 7b, and one (0.6%) to genome type 7c [Wadell et al., 1981]. Two isolates that were neutralized as serotype 3, and four isolates that were neutralized as

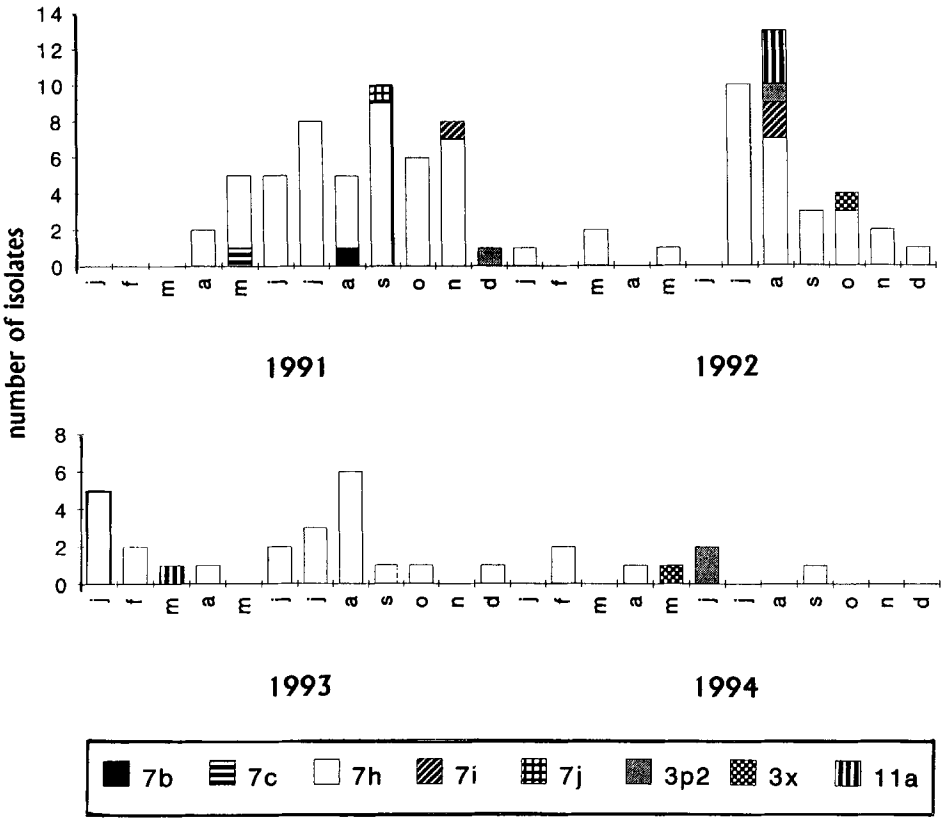

Fig. 4. Temporal distibution of genomic variants of subgenus B.

serotype 7 exhibited novel BamHI cleavage profiles. They were termed 3x, 7i (n = 3) and 7j (n = 1) according to the system proposed by Li and Wadell [1986]. The Cfo I, BamH I, and Sma I, restriction profiles of these variants are shown in Figure 1. All 101 isolates of Ad7h yielded the same digestion pattern with a set of seven endonucleases including Cfo I. Restriction profiles obtained with BcI I, BgI II, BstE II, EcoR I, Hind III, and Sma I for genome types 7i and 7j were identical to those previously reported for genome type 7h [Niel et al., 1991] (data not shown). Further characterization of genome type 3x was also carried out by restriction with BcI I, BgI II, BstE II, EcoR I, Hind III, and Xho I (Fig. 2).

Strains corresponding to genome type 7h were isolated from 17 out of the 18 fatal cases, and Ad7c was isolated from one case.

After digestion with BamHI, BgI II, BstE II, EcoRI, Hind III, Kpn I, and Sma I, 47 isolates (28.5%) were classified within subgenus C and denominated according to the system proposed by Adrian and Wigand [1985]. Thirteen of these strains were found to correspond to five new genomic variants (I-V) exhibiting either novel combinations of previously described cleavage patterns [Adrian et al., 1990] or new digestion profiles (Fig. 3). The corresponding enzyme codes are presented in Table II. Only one isolate (0.6%) obtained in July, 1992 was typed as Ad4a of subgenus E [Li and Wadell, 1988b]. The results of the genomic analysis of the 165 strains are summarized in Table III.

The temporal distribution of isolates belonging to subgenera B and C is presented in Figures 4 and 5, respectively. The chronology plots for both subgenera show an increased number of isolates between June and October (winter and early spring in the Southern Hemisphere) but no seasonality.

DISCUSSION

As recorded in previous studies [Kajon and Vicente, 1990, Kajon and Wadell, 1992; Kajon et al., 1994], a high proportion of subgenus B types (70.9%) was found in the analyzed collection of adenovirus isolates. Genome type 7h, identified for the first time in South America in 1984, represented 61.2% of all characterized genomic variants. Ad7h circulated throughout the entire 4-year period and again, in this series of cases of adenovirus respiratory infection, appeared as the genome type associated with the most severe clinical presentations. As compared to infections by other adenovirus types, infection by Ad7h was also associated with a significantly higher mortality (17/101; 16.8% vs. 1/64; 1.6%; $P < 0.001$).

The occurrence of two new genome types of Ad7 (7i and 7j) and one new genome type of Ad3 (3x) during the study period is noteworthy, although only a few isolates

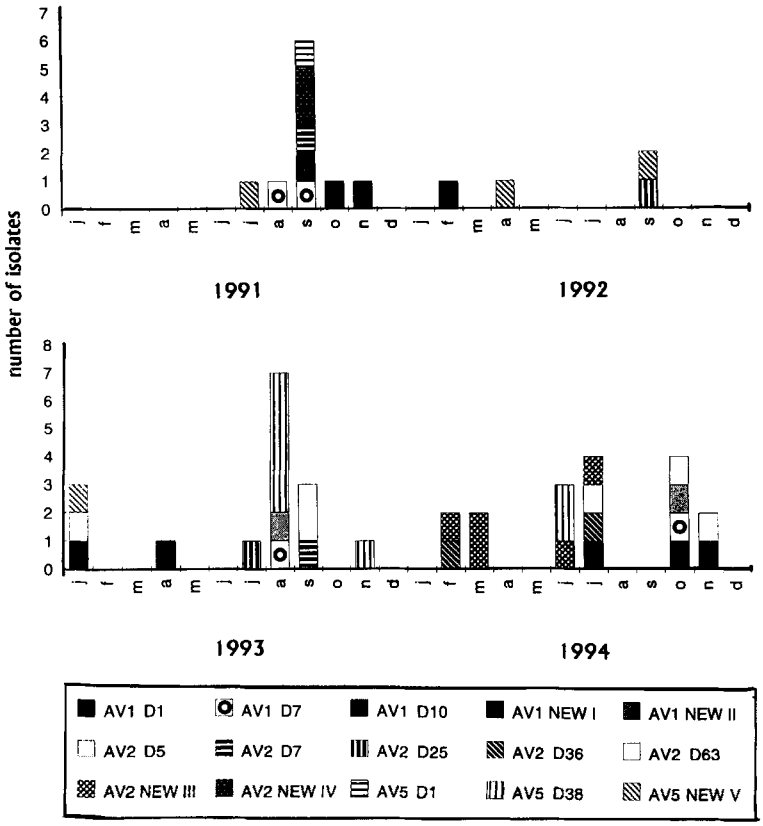

Fig. 5. Temporal distribution of genomic variants of subgenus C.

TABLE III. Genome Analysis of 165 Adenovirus Strains

Subgenus	Genome type	Number of isolates (%)	Associated fatalities	Years of circulation
B	3p2	4 (2.4)	0	91, 92, 94
n = 117	3x	2 (1.2)	0	92–94
(70.9%)	7b	1 (0.6)	0	91
	7c	1 (0.6)	1	91
	7h	101 (61.2)	17	91–94
	7i	3 (1.8)	0	91, 92
	7j	1 (0.6)	0	91
	11a	4 (2.4)	0	92, 93
C	AV1 D1	6 (3.6)	0	91–94
n = 47	AV1 D7	4 (2.4)	0	91, 93, 94
(28.5%)	AV1 D10	1 (0.6)	0	91
	AV1 NEW I	1 (0.6)	0	93
	AV1 NEW II	1 (0.6)	0	91
	AV2 D5	2 (1.2)	0	93, 94
	AV2 D7	2 (1.2)	0	91–93
	AV2 D25	2 (1.2)	0	92, 93
	AV2 D36	2 (1.2)	0	94
	AV2 D63	6 (3.6)	0	93, 94
	AV2 NEW III	5 (3.0)	0	94
	AV2 NEW IV	2 (1.2)	0	91
	AV5 D1	1 (0.6)	0	91
	AV5 D38	8 (4.8)	0	93, 94
	AV5 NEW V	4 (2.4)	0	91–93
E	4a	1 (0.6)	0	92
n = 1				
(0.6%)				

corresponded to these DNA variants. Restriction analysis with a set of eight endonucleases showed Ad7i and Ad7j to be closely related to Ad7h and to differ from it only in their Bam HI and Cfo I digestion patterns.

Isolation of four strains corresponding to genome type 7i was reported by Baumeister et al. [1993] in association with cases of pediatric acute respiratory infection admitted to a hospital in Buenos Aires, in September 1990. This variant was not identified in a sample of 65 epidemiologically unrelated Ad7 strains isolated during the same year and characterized previously [Kajon and Wadell, 1994].

The circulation of strains belonging to genome type 11a is also of interest since, to the best of our knowledge, this is the first time members of subgenus B:2 are identified in association with respiratory infections in South America. Ad11a has been isolated in China also from cases of respiratory disease [Li et al., 1991].

The use of a set of seven restriction endonucleases allowed discrimination of 15 DNA variants belonging to serotypes 1, 2, and 5. As in previous years, D1 was the predominant genome type of Ad1 [Kajon et al., 1993]. Genome types D63 and D38, also detected in previous studies [Kajon et al., 1993], were the predominant variants of Ad2 and Ad5, respectively.

The relatively higher genetic variability described for subgenus C adenoviruses by Adrian et al. [1989, 1990] was also evident among the isolates characterized in this study, as revealed by the co-circulation of several genomic variants of the same serotype and the occurrence of five new genome types.

The results of this survey indicate that Ad7h, the predominant virulent genome type of serotype 7 in the region since 1986 [Kajon and Wadell, 1994], is still an important causative agent of pediatric respiratory infections requiring hospitalization. The early onset of Ad7h infection and the severity of the resulting illness, also described in previous studies [Murtagh et al., 1993; Kajon et al., 1994], emphasize the need for rational control programs.

ACKNOWLEDGMENTS

This study was supported by a grant from the Swedish Agency for Research Cooperation with Developing Countries (SAREC).

REFERENCES

Adrian T, Best B, Wigand R (1985): A proposal for naming adenovirus genome types exemplified by adenovirus type 6. Journal of General Virology 66:2685–2691.

Adrian T, Sassinek T, Wigand R (1990): Genome type analysis of 480 isolates of adenovirus types 1, 2, and 5. Archives of Virology 112: 235–248.

Adrian T, Wigand R, Knocke KW, Schäfer G, Grundmann M (1989): Genome type analysis of adenoviruses: Isolates from one year from the Hannover area. Archives of Virology 105:89–101.

Avila MM, Carballal G, Rovaletti H, Ebekian B, Cusminsky M, Weissenbacher M (1989): Viral etiology in acute lower respiratory infections in children from a closed community. American Review of Respiratory Disease 140:634–637.

Avila MM, Salomon H, Carballal G, Ebekian B, Woyskovksy N, Cerqueiro MC, Weissenbacher M (1990): Isolation and identification of viral agents in Argentinian children with acute lower respiratory tract infection. Reviews of Infectious Diseases 12 (suppl. 8):S974–981.

Baumeister EG, Fernandez Cobo M, Savy V (1993): Deteccion de una nueva variación genómica de adenovirus 7 asociada a infecciones severas del tracto respiratorio inferior. Medicina 53:193–196.

Campbell H (1992): Acute respiratory infections are main killer under 5s. British Medical Journal 304:335.

Kajon A, Larrañaga C, Suárez M, Wadell G, Avendaño LF (1994): Genome type analysis of chilean adenovirus strains isolated in a children's hospital between 1988 and 1990. Journal of Medical Virology 42:16–21.

Kajon A, Vicente Suárez M, Avendaño LF, Hortal M, Wadell G (1993): Genome type analysis of South American adenoviruses of subgenus C collected over a 7-year period. Archives of Virology 132: 29–35.

Kajon A, Wadell G (1992): Molecular epidemiology of adenoviruses associated with acute lower respiratory disease of children in Buenos Aires, Argentina (1984–1988). Journal of Medical Virology 36:292–297.

Kajon A, Wadell G (1994): Genome analysis of South American adenovirus strains of serotype 7 collected over a 7-year period. Journal of Clinical Microbiology 32:2321–2323.

Kajon A, Vicente Suárez M (1990): Molecular epidemiology of adenoviruses isolated from hospitalized children with severe lower acute respiratory infection in Santiago, Chile. Journal of Medical Virology 30:294–297.

Leowski J (1986): Mortality from acute respiratory infections in children under 5 years of age: Global estimate. World Health Statistics Quarterly 339:138–144.

Li Q-G, Hambraeus J, Wadell G (1991): Genetic relationship between thirteen genome types of adenovirus 11, 34 and 35 with different tropisms. Intervirology 32:338–350.

Li Q-G, Wadell G (1986): Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. Journal of Virology 60:331–335.

Li Q-G, Wadell G (1988a): Comparison of 17 different genome types of adenovirus type 3 identified among strains recovered from six continents. Journal of Clinical Microbiology 26:1009–1015.

Li Q-G, Wadell G (1988b): The degree of genetic variability among adenovirus type 4 strains isolated from man and chimpanzee. Archives of Virology 101:65–77.

Murtagh P, Cerqueiro C, Halac A, Avila MM, Kajon A (1993): Adenovirus type 7h respiratory infections: A report of 29 cases of acute lower respiratory disease. Acta Paediatrica 82:557–561.

Nascimento JP, Siqueira MM, Sutmoller F, Krawczuk MM, Farías V, Ferreira V, Rodriguez MJ (1991): Longitudinal study of acute respiratory diseases in Rio de Janeiro: Occurrence of respiratory viruses during four consecutive years. Revista del Instituto de Medicina Tropical de Sao Paulo 33:287–296.

Niel C, Moraes MTB, Mistchenko AS, Leite JPG, Gómes SA (1991): Restriction site mapping of four genome types of adenovirus types 3 and 7 isolated in South America. Journal of Medical Virology 33:123–127.

Shinagawa M, Matsuda A, Ishiyama T, Goto H, Sato G (1983): A rapid and simple method for preparation of adenovirus DNA from infected cells. Microbiology and Immunology 27(9):817–822.

Vicente Suarez M, Wu E, Carrasco L, Acevedo C, Ramirez R, Peña A, Larrañaga C, Morales T (1988a): Participación viral en las infecciones respiratorias agudas bajas del lactante. Revista Chilena de Pediatría 59:353–357.

Vincente Suarez M, Wu E, Carrasco L, Torrijos J, Massu M, Vildoso J, Cantos A, Hanke MT (1988b): Deteccion viral en infecciones respiratorias agudas en niños hospitalizados. Estudio serológico. Enfermedad Respiratoria y Cirugia Toracica 4:10–14.

Wadell G, de Jong JC, Wolontis S (1981): Molecular epidemiology of adenoviruses: alternating appearance of two different genome types of adenovirus 7 during epidemic outbreaks in Europe from 1958 to 1980. Infection and Immunity 34:368–372.